SPACECRAFT STERILIZATION: IMPLICATIONS AND SUGGESTIONS

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I. INTRODUCTION

The authors are among those scientists who several years ago attempted to point out the merits, the necessity for, and the problems associated with spacecraft sterilization (Ref. 1). The idea of a numerical tolerance was suggested (Ref. 2) to accomplish two things: (1) to make evident the importance of contamination prevention and (2) to attempt to translate a value judgment of this kind into an estimate of engineering effort.

It rapidly became evident that sterilizing a spacecraft was a fairly complex problem in logistics, not dissimilar to a space flight operation. Wherever possible it is desirable to simplify operations if simplification does not interfere with the final objective. From this standpoint, heating of the spacecraft appears to have many advantages, and the higher the temperature the better the sterilization of the spacecraft is likely to be.

If the problem were that simple there would be no need for further discussion. But unfortunately microorganisms and spacecraft components have great variability in their response to high temperatures. A small percentage of microorganisms is resistant to temperatures above 100°C, and a few percent of critical spacecraft components are heat sensitive below 130°C**. In fact, if it were possible to devise an acceptable sterilization procedure that required only a terminal heating of 120°C many engineering problems would be lessened, the choice of scientific instruments would be widened, and the cost of spacecraft construction would be reduced. Consequently, spacecraft sterilization, like other logistic operations, requires flexibility, and this begins at the design.

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**An accompanying paper by Nicks and Miles shows that considerable progress has been made in the production of thermally stable spacecraft components (Ref. 3).

The design of a spacecraft system is very sensitive to the choice of environmental parameters. For a Mars landing system the pressure, temperature, atmospheric constituents, surface roughness, and average winds play a significant part in the choice of a design and the anticipated success of the mission. The current uncertainty in these parameters results in a reduced payload by a factor of at least three.

Furthermore, the proper choices of instruments for detecting life are not unaffected by the environment. But the likelihood of contamination of the planet is most sensitive to the uncertainties of the Martian environment, particularly the atmospheric components. It is known that the water content and the oxygen content of the Martian atmosphere are low. This eliminates many microorganisms as possible planetary infectors. However, if for a certainty oxygen were absent, then aerobic organisms would definitely not multiply in the Martian environment.

II. POSSIBLE INFLUENCE OF STERILIZATION ON MODE SELECTION

It is generally believed that contamination would have a greater likelihood of occurring if a spacecraft entered the Martian atmosphere but did not descend properly. The likelihood of placing an organism on the Martian soil is a function of the probability that a spacecraft will disintegrate on landing, even if the spacecraft has been sterilized to a high standard.

The probability of disintegrating a spacecraft into micron-size particles is in any circumstance not high but, since our contamination standards invoke low probabilities such as 10^{-4}, this matter requires discussion. In addition, the more severely a spacecraft is heated, the more anxiety there is about its functioning properly. Sterilization, therefore, (particularly heat sterilization) suggests minimizing the number of components and putting emphasis on landing reliability. Consequently, the entire mission design is affected by the attitude that is assumed toward sterilization. Sterilization is not a straightforward operational superposition on the mission.

There are several hundred variations of ways in which to land a spacecraft on Mars. Only a few of the more conservative approaches have received detailed attention. The most conservative is one where the entire deceleration on entry is aerodynamic; that is, the system is passive. It impacts the Martian surface at speeds up to 150 m/sec, depending upon the density of the atmosphere at the surface. With a crushable structure stroke of less than 40 cm, the accelerations on components can be kept around 5,000 g ($g = 980$ cm sec^{-2}) for a period of a few milliseconds. Almost all electronic components can be packaged to withstand this loading. Tantalum capacitors, one of those few types of heat sensitive components, have been tested to 10,000 g without loss of electrical characteristics. (The only components that have been broken were made of glass.)

This design attempt at inherent reliability against descent failure and contamination does not come without a price. It is the least efficient method of delivering weight for scientific experiments. Payloads delivered to the surface of Mars in this manner would weigh between 5 and 100 kg, with 20 kg being a reasonable mean number.

A more efficient method of landing on Mars would be to employ a parachute descent system. The velocity before parachute deployment would be roughly 300 m/sec. The impact stroke would have to be shortened if the system were to enjoy any scientific weight advantage over the passive aerodynamic technique. If the parachute did not open, the accelerations on the components would be greater than the previous mode by approximately a factor of 10, that is, 50,000 to 100,000 g. These are approximately the accelerations of bullets. The metal parts should not shatter, but this statement cannot as yet be made for all that goes into a spacecraft.

Soft-landed payloads have the prospect of being the most efficient at landing scientific payload weights. However, the system would suffer accelerations of several hundred thousand g if the retro system did not ignite.

These estimates of the accelerations on impact assume a hard, flat, or slightly concave or convex surface. If the surface of Mars is soft, then no breakage would occur for any of the systems just discussed. On the other hand, if they hit dead center the point of a structure as sharp and hard as the tip of the Washington Monument none of the systems would survive.

The probability of mission success and spacecraft disintegration is directly related to the probabilities of the Martian terrain.

Ironically, the small, more conservative systems, which by virtue of their design are less likely to pollute Mars, are also the easiest to terminally heat sterilize.

III. RE-EXAMINATION OF THE DEATH CURVES

It is generally assumed that the equation governing the rate of change of viable microorganisms is

$$\frac{dN}{dt} + KN = F \qquad (1)$$

where N is the number of viable organisms, K is the death rate, and F is the source rate of new organisms from the outside. Equation (1) is a good approximation in most circumstances where N is between 10^6 and 10^2. This equation is a rough approximation to the rate of killing of microorganisms by any means. The constant K, however, must be determined experimentally.

There are some serious questions that must be raised in connection with using Eq. (1) as a basis for operational action. (See Appendix 1.) First, there is considerable scatter in the data that supports Eq. (1) below $N = 10^2$. The problem is an inevitable one of sampling error at these low numbers.

Second, below N = 1, there is not necessarily any reason to believe that Eq. (1) applies at all. This can best be described by the diagram in Fig. 1. The number of microorganisms (colonies or infective centers) is plotted vertically on a logarithmic scale. Let us assume that our laboratory data give us a reasonable exponential death curve shown as Type I. Let us suppose that, within the test data, there were some highly heat resistive strains. Type II, for example, makes up only 1/100 of 1% of the original sample, but if it is four times as resistant to heat it will have the same probability of showing up at the N = 1 level as Type I. Figure 1 is purely illustrative. Figures 2, 3, 4 and 5 demonstrate that these comments are not hypothetical. All these figures show that a small number of heat resistant cells can, and often do, contribute disproportionately to the final surviving population.

We note that any given sterilization heat treatment, such as 135°C for 24 hr, must assume the virtual absence of any organisms capable of withstanding this temperature.

At first glance this may seem disquieting. But it does not mean that low probabilities of contaminating Mars cannot be achieved by other means. It merely illustrates that, below the level of practical detectability ($N \leq 1$), the extrapolation of the thermal death curve is based on faith. The primary merit of heat sterilization is its usefulness down to the $N \simeq 1$ level.

As N goes below $N \simeq 1$, the problem has a different philosophical and technical basis. Above $N = 1$, there are sufficient experimental data to justify some kind of statistics. Below $N = 1$ ($P \ll 1$), the problem is concerned with probabilities which as yet are not based upon any frequency data. In theory it would be possible to base these probabilities upon laboratory data, but for $P \simeq 10^{-4}$ this would require thousands of tests; this, for the time being, is not practical.

The constant K, we recall, is determined experimentally by heating initial populations of approximately 10^6. Figure 1 illustrates that if the spacecraft had an initial load of only 10^2 organisms (of the type for which the constant K was determined) then it is reasonable to extrapolate four orders below $N = 1$.

This illustrates another point even better. If the initial spacecraft load is $N = 10^7$ (one order of magnitude higher than the laboratory death curve data), this additional load cannot be necessarily compensated for by increasing the length of the heating one death time (1/K) unit as sometimes has been suggested.

These points have been mentioned previously principally by Koesterer (Ref. 4) and Bruch (Ref. 5).

IV. INTERNAL CONTAMINANTS

In order to determine what is a necessary and sufficient terminal sterilization procedure of a fully assembled and tested spacecraft, we need to estimate the microbial load at that time. The first estimate of this kind was given by G. Hobby (Ref. 6), who suggested 10^9 on the basis of some sampling in the Ranger spacecraft assembly area over a period of 90 days. D. Portner's data (Ref. 7) suggested that this be revised downward to between 10^6 and 10^7 organisms on the basis of some sampling over a 33-day period in a microbiological laboratory. These early estimates were given as starting points for initiating a sterilization program. They highlighted the fact that, in terms of numbers of organisms, the greatest fluctuation (loss or gain) was in the spacecraft assembly process. A very clean or sterile assembly facility would appear to have some merit.

However, it is difficult to determine the level of cleanliness required of a spacecraft assembly facility until something is known about the level of contaminants internal to spacecraft components. If our internal contamination load is 10^p, then the assembly process certainly need not be any cleaner than another 10^p, because a factor of two is not significant if a terminal heat sterilization process is to be used.

The estimate of the internal contaminants depends, in turn, upon the nature of the spacecraft system and its components. Mariner IV is a useful example of a fairly complex system. It can be used as a basis for estimates of future Mars landing systems. It weighs approximately 250 kg, of which 150 kg is metal structure and parts which are intrinsically sterile or could be heated to high temperatures. The remaining 100 kg contains over 35,000 electronic piece parts. The scientific subsystem weighs about 35 kg and contains half of these piece parts. Over 11,000 of these parts are in the scientific data system, which weighs less than 20 kg. Certain electronic components, key to spacecraft systems, are sensitive to temperatures above 120°C as is the battery system. Consequently, most of our discussion will deal with electronic parts.

If the preceding example is a guide, we can see that heat sensitivity is most likely to be found in the scientific subsystems.

Fortunately, most electronic components are not extremely heat sensitive. Transistors, diodes, and resistors can be selected to withstand 135°C (Ref. 8). The most significant exceptions are the tantalum

solid-state capacitors. They are not often employed in subsystems, but their high capacity makes them irreplaceable. For example, in a 2.5-kg instrument designed to measure the atmospheric constituents, there are 350 electronic piece parts. Of these, 25 are tantalum capacitors, of which 4 to 6 are critical.

We next need an estimate of basic cleanliness of electronic components. Data on the extent of internal contamination of electronic components were first published by Phillips and Hoffman (Ref. 9). They determined the number of items with internal contamination among 150 assorted types. The surfaces were first sterilized with ethylene oxide; each component was then pulverized aseptically and used to inoculate a flask of broth. After incubation, the flasks were scored for growth or no growth. In this way 21, or 14%, of the items were contaminated. Subsequently, these authors tested additional components in a similar manner. According to Report No. 13-61, they found that approximately 7% of electronic components were contaminated prior to a final batch of 218 sent them by G. Hobby. Of these, half was heat sterilized at 125°C for 27 hr. The other half was surface sterilized as discussed above. Neither group showed growth. We can conclude, therefore, that less than 4% of electronic components are detectably contaminated. Equally significant is the fact that no tantalum capacitors were found to contain internal contaminants. This is not surprising because they are manufactured above 100°C.

We can partially summarize this by stating that the 35,000 electronic piece parts of Mariner IV probably contain less than 1,400 detectable infective centers. By heating the heat resistant components only, for a short period prior to their assembly, the total number could have been kept to less than 70 detectably contaminated components (the number probably closer to 0 than to 70).

Most of the parts of a vidicon tube are made at 300°C. The coating of the sensing material is done at 150°C. We can expect the tube to have a very low load. The tube can take up to 100°C with only 5% degradation. Time of heating is an important factor, however, because the deposited material on the photocathode begins to diffuse.

The battery plus the vidicon tube probably contains less than two infection centers--considerably less than the upper estimate of the number inside the electronic piece parts.

There is, as yet, no way of determining what kind of contamination exists inside electronic components. Are these single organisms or groups of them, and what is their distribution? The statistically least bias estimate is to assume a Poisson distribution until more information is available. There is one other reason to suspect that this distribution is applicable. The Poisson distribution is known to apply to organisms floating in the atmosphere (Ref. 10). For a Poisson distribution with an average of 0.04, the probability of a single component having more than one contamination center is only 0.0008.

It is legitimate to argue that a certain fraction of the microorganisms escape detection. L. Reed at JPL, Pasadena, California, has found it difficult to recover 1% of the spores inoculated in various cementing materials. Whether the recovery technique is poor or whether encapsulating is lethal to most spores cannot be determined. That they will not escape subsequently by diffusion can be shown by calculating the diffusion rate of a 1-micron organism in a substance as soft as wax.

$$t = \frac{\overline{X^2}}{2D}$$

where D is the diffusion coefficient, $\overline{X^2}$ is the mean square of the distance diffused, and t is the time.

$$D = \frac{RT}{6\pi\eta a}$$

where T is the absolute temperature of 300°K, a is the radius of 1 micron, and η is the viscosity of wax of 4.7×10^6 poise. If we take X to be a distance of 1 micron, the time is 40 yr. Thus, an organism trapped inside a wax component several microns in diameter would, on the average, require several decades to emerge. Most solids have viscosities that are a few orders of magnitude larger than the value used here.

It has been suggested that erosion from wind-driven sand might release organisms from the interiors of components. Field data obtained by Prof. Robert Sharp (Ref. 11) indicate that this kind of erosion would be negligible. A lucite rod was placed vertically in the wind-blown Coachella

Valley of California. The rod's harder outer surface was eroded in varying amounts by hard driving sands depending upon the distance from the ground. The point of maximum erosion was 25 cm from the ground; the amount was approximately 0.05 mm in 9 yr. Softer materials eroded appreciably in this period. The erosion of hard rock was not detectable. It is well known, of course, that metal posts, wires, and thin sheets of soft metal that protect the bases of wooden posts show no sand erosion over periods of more than 20 yr.

V. FALLOUT CONTAMINATION

Let us now consider the potential contaminants on a spacecraft that would accrue to fallout during electronic and spacecraft assembly. We shall continue to use Mariner IV as a discussion model, and then finally scale down and up accordingly for the early and later Mars landing systems.

Our object in this section is to determine what procedures are necessary to limit the microbial fallout on the spacecraft so that they add no more than the equivalence of a factor of two to internal loading. We are referring to the experimental evidence (Ref. 4) that organisms trapped in a variety of solids show greater heat resistance than do the same organisms not so entrapped, by approximately a factor of two.

We must remember that much of the microbial fallout can be eliminated by ethylene oxide sterilization during and after assembly. We are only concerned with the fallout that surface sterilization will not remedy. It is estimated that the open area, that was later closed up on Mariner IV, is approximately 50 ft^2. We shall assume it is 100 ft^2 (9×10^4 cm^2). Thus, 90 FT is the total fallout contamination, where F is the average fallout flux per $10^3 cm^2$ and T is the total "effective" exposure time to fallout. We now have four parameters which can eventually be varied: the number of piece parts, the exposed area (which is related to the piece part number), the flux, and the time.

The most important is the character of the flux rate. Let us examine some preliminary fallout data gathered by Portner at an East Coast aircraft factory, electronic assembly, and clean room areas in Baltimore, Maryland; and by McDade and Irons at a West Coast aircraft facility in El Segundo, California.

It should be noted that the number of microorganisms per cubic foot of air in the clean areas is an order of magnitude less than in the factory area. Also, the fallout on horizontal surfaces is an order of magnitude less in the clean areas than in the factory area.

If we designate the flux rate as F, the bacterial concentration in air as n per cubic meter, and the fallout velocity as V, then

$$F = nV \text{ per square meter per hour}$$

where, for both the clean areas and the factory area, Portner's data show that V varies between 0.2 and 1 cm/sec. This corresponds to the setting velocities in still air of particles 6 to 12 microns in diameter. Presumably, the filtering system to clean areas removes almost four orders of magnitude of the total dust content of air, yet the microbial count is lower by only an order of magnitude. The difference is probably due to human activity in the area.

Microorganisms/Meter^3 (1 cubic ft = 2.7 x 10^3 cm^3)

Area	Aerobes	Anaerobes
Factory	133 < 142 < 163	29 < 41 < 48
Cleaning	9 < 19 < 22	0.7 < 2.6 < 5.2
Assembly	12 < 15 < 24	1 < 3.7 < 5.4

Microorganisms/Square Meter/hr Averaged Over Eight 1-hr Samples in 8 wk

Area	Aerobes	Anaerobes
Factory	2270 < 2570 < 3080	250 < 460 < 750
Cleaning	140 < 230 < 370	20 < 80 < 250
Assembly	100 < 170 < 300	10 < 60 < 210
Average of cleaning and assembly	120 < 200 < 250	30 < 70 < 180

The upper and lower limits bracket the 95% confidence level for counting errors. The middle number is the computed average.

Statistics are based upon data taken by D. Portner at East Coast Co., Test No. 10-64, February 3, 1964.

Approximate Aerobic Contamination After 11-wk Exposure Per Square Meter

Area	Aerobes	Aerobic Spores After Heat Shock	Anaerobes	Anaerobic Spores After Heat Shock
Factory	67,000	30,000<	2000	~1200
Cleaning room	8500	800	2250	100<
Assembly	1900	1800	~350	150

The cleaning room and assembly area seem to have stable spore populations of between 2000 and 4000 per square meter over a period of a year. The factory area population rose to 10^5 per square meter in 17 wk, but later stabilized to approximately half that value over a year. The flux rate in a factory would clearly have a more variable statistic and be considerably more dependent upon the season.

These data are consistent with those of McDale and Irons taken at the "Surveyor" Facility on the West Coast.

Accumulated Spores on Metal Surfaces Within the West Coast Facility

Exposure Time, wk	Average Over 14 Sites Per Square Meter*	Average Over 13 Sites Per Square Meter+
1	1300 < 1750 < 2260	1040 < 1440 < 2000
2	1720 < 2230 < 2740	1300 < 1840 < 2110
4	2940 < 3470 < 4000	2220 < 2700 < 3180
8	Not Applicable	1960 < 2530 < 3210

*95% confidence levels of the counts bracket the averages.

+Excludes one consistently dirty site.

Data acquired by J. J. McDade and A. S. Irons, JPL, Pasadena, California (Interoffice Memo No. 2945, dated January 22, 1965).

After 1 wk, about 13,000 spores accumulated per square meter in the East Coast factory. This is a minimum of 80 per square meter per hour, 100 to 120 being more probable. The accumulation in the cleaning room and assembly area were about 750 and 4000, respectively, that is a flux rate of between 5 and 20 spores per square meter per hour. We note that it is about 10 spores per square meter per hour in the West Coast aircraft facilities.

Coarse as these data are, they provide an opportunity to estimate the natural death rates of bacteria and spores, from the expression

$$KN = F \text{ per square meter per hour}$$

(See Appendix, Eq. 6.)

The vegetative cells have a death rate of several percent per hour; however, the death rate of the spores is of greater concern to us.

$$K = \frac{F}{N} = \frac{5 < 10 < 12}{1300 < 2000 < 3200}$$ West Coast Facility

$$\boxed{\frac{1}{170} > K > \frac{1}{640} \text{ per hour}}$$

$$K = \frac{F}{N} = \frac{\frac{1}{2} \text{ to } 1}{75 \text{ to } 350}$$ East Coast Clean Room

$$\boxed{\frac{1}{75} > K > \frac{1}{700} \text{ per hour}}$$

$$K = \frac{F}{N} = \frac{\frac{1}{2} \text{ to } 2}{50 \text{ to } 400}$$ East Coast Assembly Area

$$\boxed{\frac{1}{100} > K > \frac{1}{800} \text{ per hour}}$$

K values of between 1/200 and 1/400 seem typical of most of the data.

The fallout rate and the accumulated spore count are higher in the East Coast factory area. It is easier, therefore, to make a statistical estimate of K for this facility. After 8 wk, the final population had an average of approximately 5×10^4 spores per square meter. Using Eq. (6), Appendix 1, we fit a curve to the data as they accumulated during the first 8 wk. This gave a death rate of $K \simeq 1/500$ per hour.

Since these are the death rates at approximately 20° C, we can ask what might they be at 80° C. The electronics of the spacecraft are being designed to function up to 85° C. Heating the electronics up to 70° C or 80° C should not be a problem. In general, biological reactions increase by a factor of two every 10° C. It is not unreasonable to suspect that this would also apply to the death rate of spores on a metal surface. At 80° C, the death rate would be increased by a factor of 64.

$$K_{80°} = 64K_{20°} = \frac{64}{400} = \frac{4}{25} \text{ per hour}$$

During the working week of 40 hr, the spacecraft subassemblies would accumulate between 200 and 800 spores per square meter. If the units were heated to 80° C for the weekend, this load would be reduced to about 2×10^{-2} spores per square meter. These numbers should be regarded with some caution, however. A few unexpected factors of 2 all going the wrong way could nullify the idea. Nevertheless it is suggestive and deserves some additional investigation both theoretically and experimentally.

It might be asked how many of the spores that accumulated at the East Coast factory were of a more resistant type, say Bacillus Coagulans. We estimate the death rate of Bacillus Coagulans on paper as 5.0×10^{-5} per hour at 20° C. (See Appendix 2.) We divide the spores into two types: one that has a relatively high death rate of $K_1 = 1/200$ per hour and Bacillus Coagulans (K_2). If the total flux F in the Eastern factory is approximately 100, then

$$F \cong 100 \text{ [illegible]} F_1 + F_2 \text{ per hour per square meter}$$

$$N \cong 50,000 = N_1 + N_2 \text{ per square meter}$$

$$N = \frac{F_1}{K_1} + \frac{F_2}{K_2}$$

from Eq. (6), Appendix 1. The flux of Bacillus Coagulans (B. C.) is then

$$F_2 \simeq 1.5 \text{ B. C. per hour per square meter}$$

or about one organism in 66. The flux in the clean room is about one order of magnitude lower, or

$$F_2 < 0.2 \text{ B. C. per hour per square meter}$$

These are very rough estimates, but it is difficult to do better with the currently available data. The estimates are encouraging in that the frequency of resistant spores is low. This is precisely what was hoped for. The concept of clean and sterile assembly is within the realm of practicality.

VI. THE ASSEMBLY PROCESS OF SUBSYSTEMS

Each subassembly will have its own problems which can only be surmised until a mission profile is actually selected. Consider the atmosphere analysis mentioned earlier. Its exposed surfaces (that will later be closed) are less than 10^3 cm^2. This unit has 4 to 5 sensitive components. The remainder of the unit can be heated to 135° C. The amount of fallout contamination would be restricted to the length of time required to solder in these four components. This would be less than 2 hr because the instrument can be designed for this special procedure. In this period, fewer than four spores (1/10 resistive spores) would fall on the entire instrument, and the probability that they would be trapped would be very small because the combined areas of four components would be less than a few square centimeters. The engineer could wear sterile gloves and mask, and work under an ultraviolet lamp as is done in surgical rooms. This particular unit could then be heated again to 115° C, but surface sterilization would probably be sufficient.

VII. EVALUATING CONTAMINANTS AND THE CORRESPONDING TERMINAL STERILIZATION

The evaluation of the locations and nature of spores inside electronic components determines the terminal heat cycle and the quality of the clean facility that it is practical to employ during spacecraft fabrication and test. There are three ways to evaluate the problem:

(1) Organisms detected inside components are accepted as being representative of the actual number density. Furthermore, this is the maximum number that could be theoretically freed on the surface of the planet if the probe were only surface sterilized. In this case one internal contaminant is equivalent to approximately 10 to 10^2 surface contaminants if the latter are assumed to be two to three times less heat resistant.

(2) Organisms detected inside components represent only one in 10^2 or 10^3 of the actual count; that is, current detectability techniques are not acceptable as a criterion for estimating the microbial load before terminal heating. In this case each counted internal contaminant is equivalent to 10^3 to 10^5 fallout contaminants.

(3) All internal contaminants are secure inside their respective components even in case of a malfunction and hard impact on the planet's surface. The surface load will then determine the terminal heating cycle.

For Case (1), a clean room facility slightly better than the one at the East Coast would be adequate. An adequate terminal heating cycle for a Mariner IV type spacecraft would be (on the basis of current information) 30 to 40 hr at 115° C, assuming the 35 to 3, 500 trapped organisms are no more resistant than Bacillus Coagulans, and that it is legitimate to extrapolate the last four orders of magnitude.

For Case (2), a facility such as that on the West Coast would be adequate. This terminal heating cycle could be as low as 45 hr at 115° C if 98% of the electronic components were preheated at higher temperatures, and 55 hr at 115° C if there was no preheating, again assuming the

organisms have the heat resistance of Bacillus Coagulans entrapped in dental inlay materials.

For Case (3), the better the clean facility the lower the required heating cycle. Utilizing the techniques of periodic cleaning suggested earlier in conjunction with a clean room facility similar to the one on the East Coast, the heating cycle could be as low as a few hours at 115° C.

A very high-quality clean facility,in conjunction with heat sterilization, will probably be required for automatic life detection spacecraft systems. The life detection program would be seriously limited if substrates and other sterile but highly sensitive materials cannot be inserted into the payload after terminal heating. If the process of insertion did not expose the unit more than a matter of seconds, it would be possible to meet the currently suggested standards of sterility. If the assembly process requires minutes,then it could be performed under strong ultraviolet lamps with adequate protection for the personnel.

APPENDIX 1

PROBABILISTIC BASIS OF EXPONENTIAL KILL CURVES

In order to understand the basis for comments on the kill curve data, it is useful to review the probabilistic basis for Eq. (1). In this we must envisage a population of microorganisms which, at some temperature, are subject to random sterility in the sense that the probability of any individual losing viability in time Δt is $K\Delta t$. It then follows that individual lifetimes x have the negative exponential distribution

$$f(x) = Ke^{-Kx}; \qquad 0 \leq x \leq \infty \tag{2}$$

It can also be shown that the probability of there being N individuals at time t is

$$P\eta(t) = \binom{N_0}{\eta} e^{-\eta Kt}\left(1 - e^{-Kt}\right)^{N_0-\eta}; \qquad 0 \leq \eta < N_0 \tag{3}$$

where N_0 is the population at time $t = 0$.

The mean number N(t) is given by

$$N = N_0 e^{-Kt} \tag{4}$$

which is a solution of Eq. (1) for $F = 0$. The variance is

$$\sigma^2 = N_0 e^{-Kt}\left(1 - e^{-Kt}\right) \simeq N(t) \tag{5}$$

For $t \gg K$, $e^{-Kt} \ll 1$. We see that for $N \simeq 10^{-4}$, $\sigma = 10^{-2}$, which demonstrates how difficult it would be to statistically verify values of N much less than 1.

If the source function F in Eq. (1) is the mean microbial fallout from the air, then it is the mean value of a Poisson random variable. If

the initial population $N_0 = 0$, then the later population has a negative binomial distribution with mean

$$N(t) = \frac{-F}{K}\left(e^{-Kt} - 1\right) \tag{6}$$

which is a solution of Eq. (1).

$$N(t) \sim Ft; \qquad t < \frac{1}{K}$$

$$N \sim \frac{F}{K}; \qquad t \gg \frac{1}{K}$$

APPENDIX 2

The death rate at two temperatures T and T + ΔT are related by the equation:

$$K_{T+\Delta T} = K_T \exp\left[\frac{a\Delta T}{2T(T + \Delta T)}\right] \tag{7}$$

where a is a characteristic coefficient of the reaction.

If the death rates are known at two temperatures, say 115° C and 125° C, then:

$$a = \frac{2(388)\ 398}{10} \ln\left(\frac{K_{125}}{K_{115}}\right)$$

For most reactions in the 115- to 125-deg range

$$K_{125} \simeq (2 - 2.3)K_{115} \text{ per hour}$$

Thus

$$K_{20} \simeq K_{125} \exp\left[-\frac{(388)(105)}{10(293)}\right] \ln\left(\frac{K_{125}}{K_{115}}\right)$$

$$K_{20} \simeq K_{125}(e^{-10.8}) \simeq \frac{K_{125}}{5.1 \times 10^4} \simeq \frac{1}{22,000} \text{ per hour}$$

Therefore

$$D_{20} = 5.1 \times 10^4 D_{125}; \quad D \leq 1 \text{ hr} \tag{8}$$

For Bacillus Coagulans on paper strips,

$$D_{20} \simeq 5.1 \times 10^4 \text{ hr} \sim 9 \text{ yr}$$

CONCLUSIONS AND RECOMMENDATIONS

1. The objectives of spacecraft sterilization are twofold. The first is to avoid confusing our life-detection experiments; obviously, any spacecraft which lands on Mars in order to search for microbial life must itself be free of microorganisms.

2. The second objective is to prevent contamination of Mars with terrestrial organisms which, by altering the natural ecology of the planet, could cause an irreparable loss to science. Terrestrial organisms cannot multiply under Martian conditions as we know them, although many can survive under these conditions in a dormant state. Nevertheless, we cannot at present ignore the possibility that local environments capable of supporting terrestrial life are to be found on Mars.

3. The problem of spacecraft sterilization cannot be solved (in the sense of a 10^{-4} probability) by simply heating the entire spacecraft and its contents to 135 deg for 25 hr, as has been proposed. Although most components can withstand this treatment and considerable progress is being made (as discussed by Nicks and Miles in Ref. 3), there are a number of critical parts, such as batteries and tantalum capacitors, which cannot take these temperatures. In addition, it is likely that certain scientific experiments will not survive this treatment, although their sterility can be assured by other methods. The mechanical stresses caused by thermal gradients in a large spacecraft pose another problem which we have not discussed here. From an engineering standpoint, a 10- or 15-deg lowering of the terminal sterilization temperature would be advantageous.

4. An examination of existing data on the load of bacteria carried within electronic components shows that such components are very clean from a bacteriological standpoint. We recommend that further studies be conducted to determine the statistical distribution of microorganisms in electronic parts and to determine the nature of the organisms--e.g., aerobes or anaerobes, heat resistant or heat sensitive. The last point is of particular importance, since a consideration of standard procedures for measuring thermal death rates of bacteria shows that they may fail to assess correctly the number of survivors following heat sterilization.

5. Available data on the microbial fallout rate in industrial clean rooms suggest that the insertion of sterile subassemblies into a previously sterilized spacecraft can be accomplished with a negligible contamination hazard. The adoption of this type of procedure would go a long way toward accomplishing the goal of spacecraft sterility without endangering spacecraft reliability.

6. The diffusion rates of bacterial spores trapped inside of solid components are entirely negligible. This means that such trapped spores cannot reach the outside unless the components are broken. In addition, field tests have shown that erosion caused by wind-blown sand is extremely slow for lucite, and for harder materials it would probably be negligible. These facts may be useful in connection with certain components which are normally sterile, but which cannot be heated.

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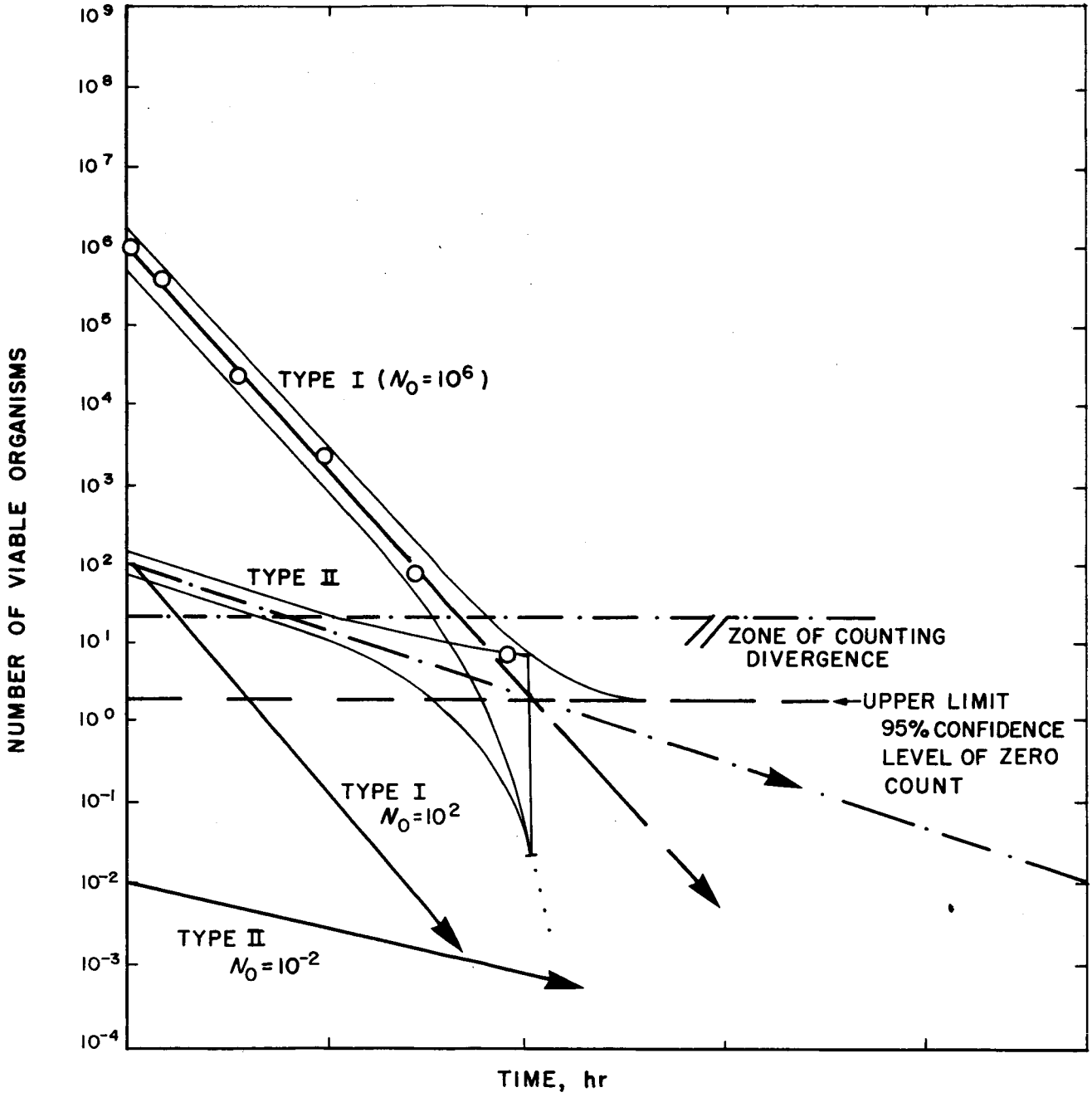

Fig. 1. WHY 10^{-4} PROBABILITY OF STERILITY REQUIRES 4 ORDERS OF MICROBIAL REDUCTION

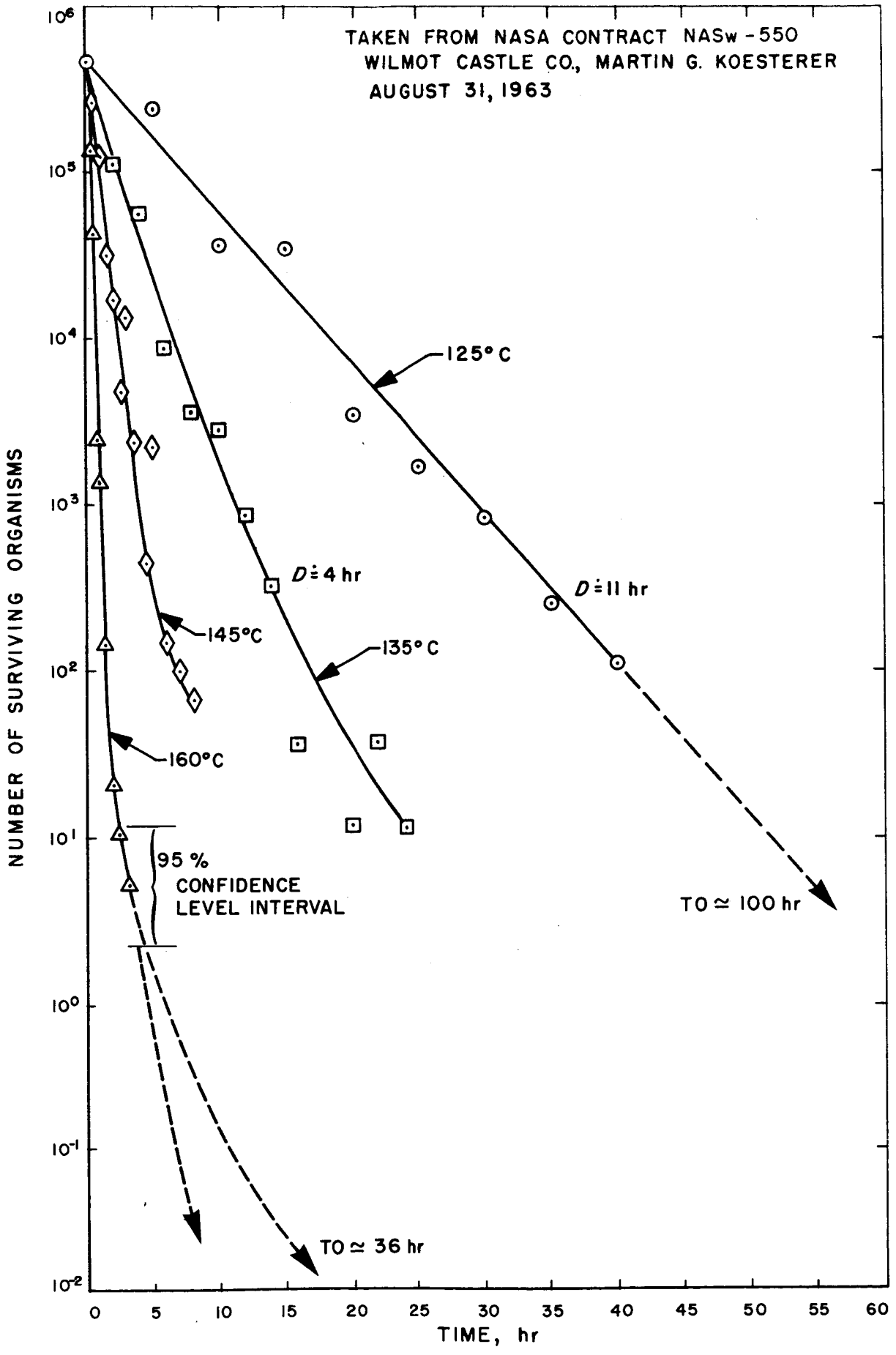

Fig. 2. DRY HEAT SURVIVOR CURVES FOR 0.1-g SAMPLE OF DRY FG SOIL INOCULATED WITH SPORES OF SOIL ISOLATE 541

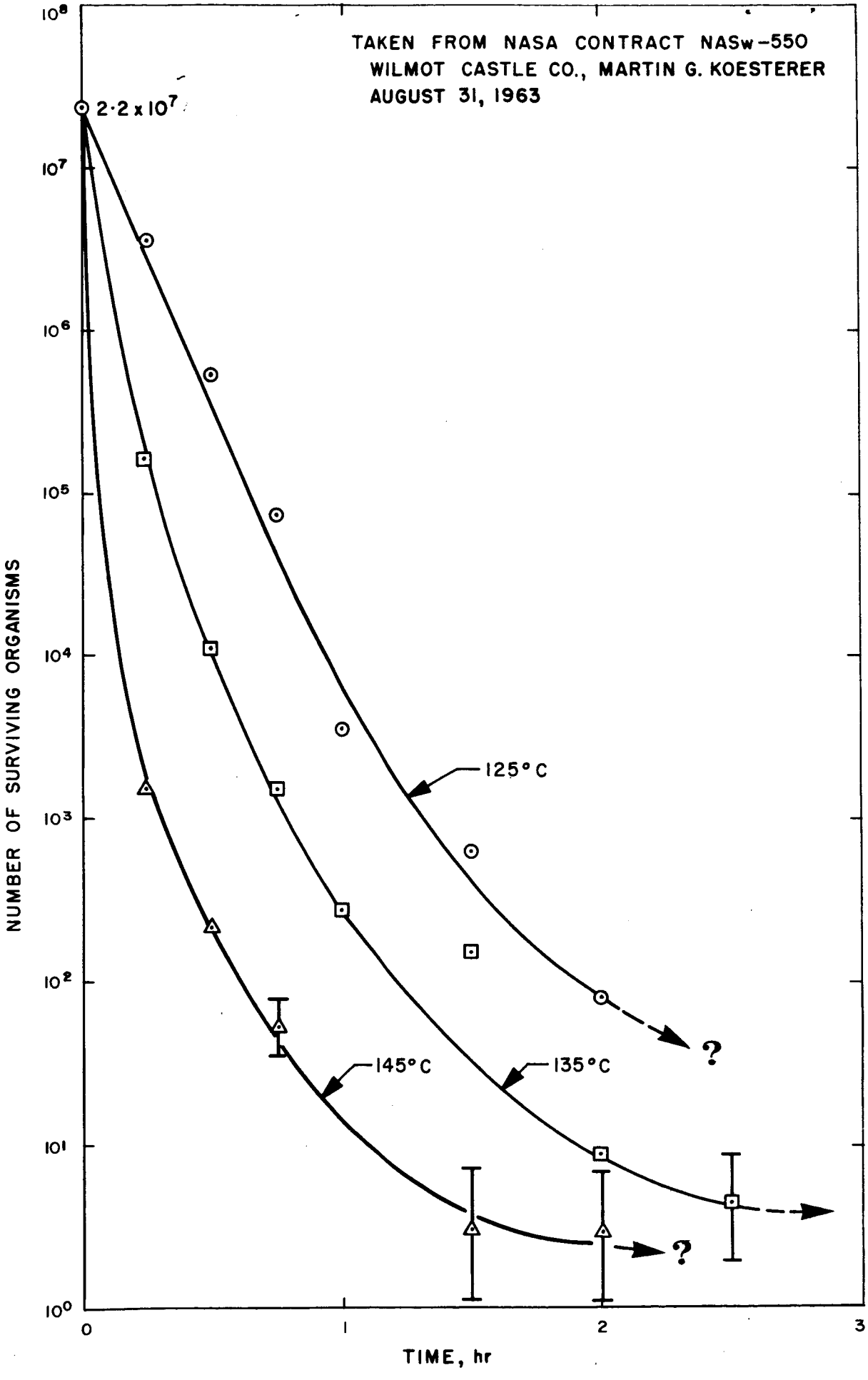

Fig. 3. DRY HEAT SURVIVOR CURVES FOR 0.1-g SAMPLE OF DRY FG SOIL INOCULATED WITH SPORES OF *BACILLUS COAGULANS*

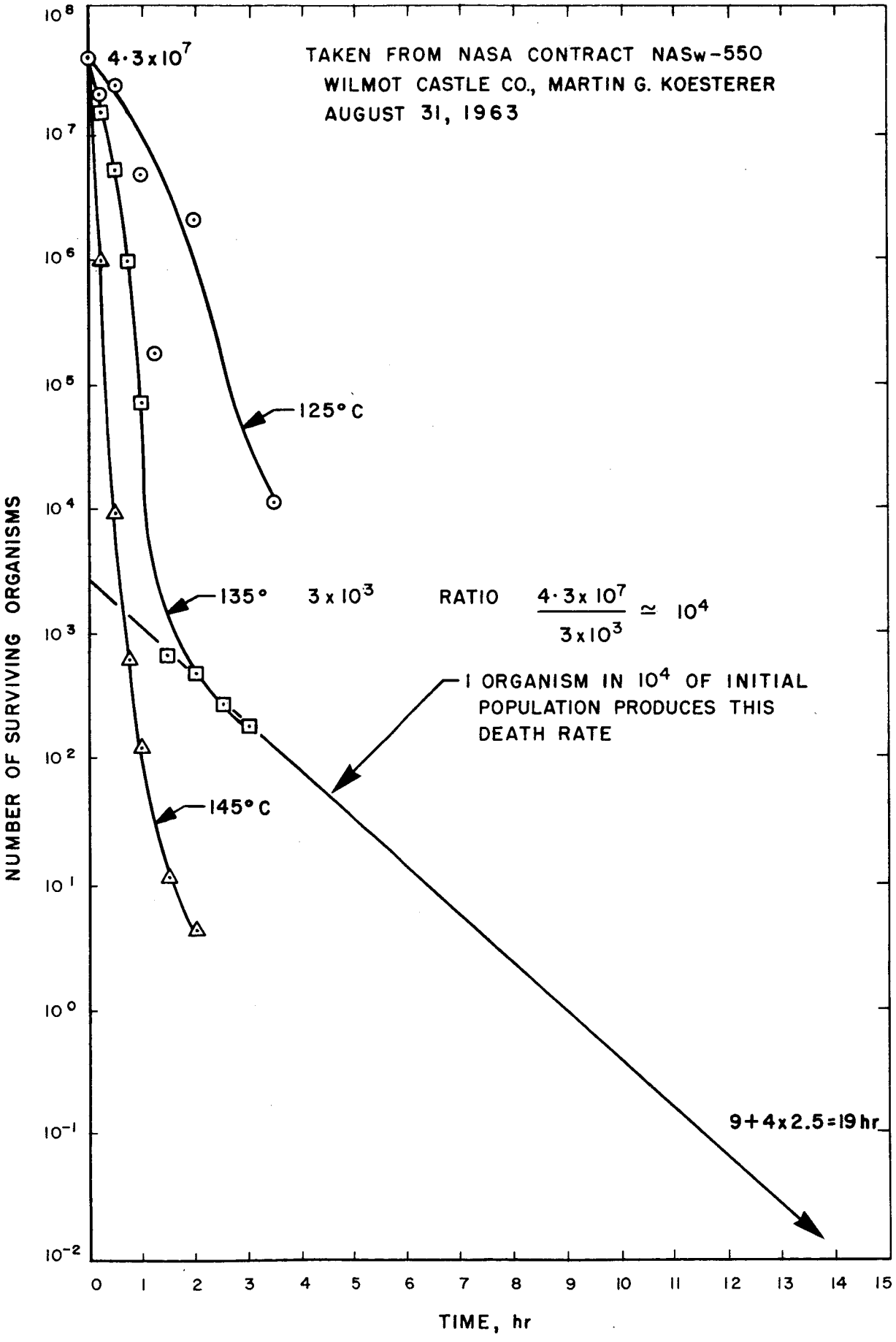

Fig. 4. DRY HEAT SURVIVOR CURVES FOR 0.1-g SAMPLE OF DRY FG SOIL INOCULATED WITH SPORES OF SOIL ISOLATE 69C

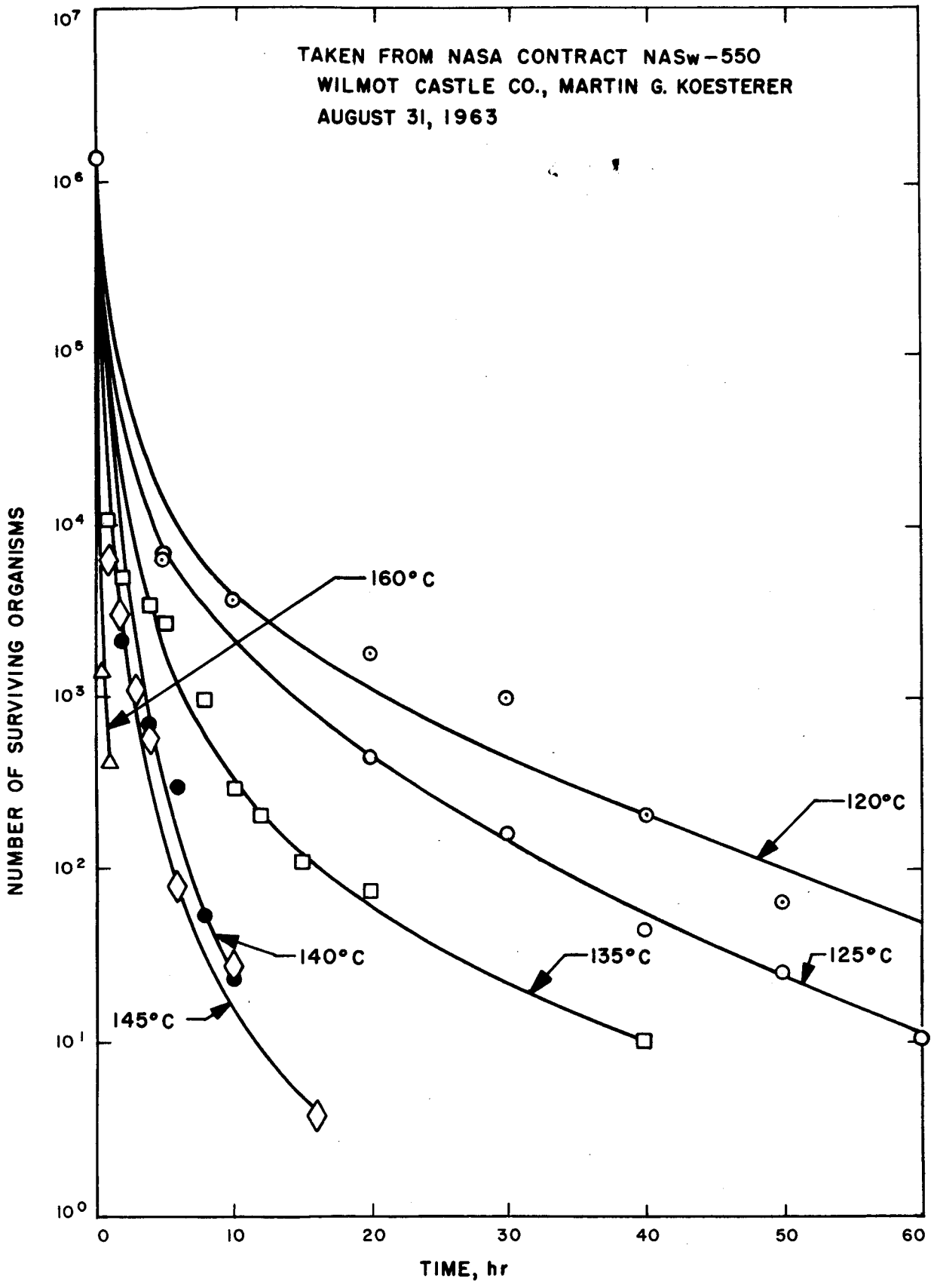

Fig. 5. SURVIVOR CURVES OF ORGANISMS IN 0.1-g SAMPLE OF DRY FG SOIL TO DRY HEAT IN THE TEMPERATURE RANGE 120–160°C